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Table S1. Bulk seed composition of selected maize genotypes, mature dried kernels.

	% starch	% oil	% protein	% fiber
W64A	57.6	4.4	17.2	n.d.
su1 in W64A	30.6	6.2	20.3	n.d.
bt1 in W64A	31.0	7.6	19.9	n.d.
bt2 in W64A	24.4	7.7	20.7	n.d.
pgd3-umu1/+, normal kernel	52.5	3.4	10.7	2.4
pgd3-umu1/+, mutant kernel	38.3	1.9	11.0	4.3
pgd3-umu2/+, normal kernel	50.7	4.1	10.0	2.0
pgd3-umu2/+, mutant kernel	38.4	1.3	10.2	4.6

Kernel composition for W64A, *su1*, *bt1*, and *bt2* are from Spielbauer et al. (2009). Kernel composition of *pgd3* mutants was from mature kernels ground to a fine meal with a coffee grinder. Moisture, protein, fat, and fiber were determined by Water Agricultural Laboratories (Camilla, Georgia, USA) according to AOAC method numbers 930.15, 968.06, 920.39, 962.09, respectively. Starch was determined as described in Spielbauer et al. (2009).

Table S2. Dry weights (mg/kernel) of tissues dissected from mature maize kernels.

		Heterozygous self-pollination		Translocation uncovering cross		
Locus	Tissue	Normal	mutant	normal	uncovered endosperm	uncovered embryo
pgd3	Kernel	243.7	31.9	233.5	15.2	208.4
	Embryo	30.3	0.9	28.3	3.8	0.2
	Endosperm	197.4	22.8	187.5	3.8	188.9
	Pericarp	16.0	8.2	17.7	7.6	19.3
dek11	Kernel	190.9	11.3	214.4	22.7	180.1
	Embryo	21.4	0.0	27.5	8.9	2.3
	Endosperm	143.9	2.1	173.8	6.1	162.9
	Pericarp	25.6	9.2	13.1	7.7	14.9
bt2	Kernel	223.4	133.9	182.5	96.0	N/A
	Embryo	23.9	22.0	22.3	22.5	N/A
	Endosperm	179.5	88.6	144.0	54.8	N/A
	Pericarp	20.0	23.3	16.2	18.7	N/A

Average dry weights were calculated from 10-60 kernels soaked overnight in water, dissected, and lyophilized for two days.

Table S3. Endosperm composition of *pgd3* mutant and normal kernels from segregating ears.

		pgd3-umu1		*pgd3-umu2*	
Stage	Constituent	mutant	normal	mutant	normal
20 DAP	% solubles	20.3±4.3	8.9±1.8	28.8±1.8	10.8±0.1
	% starch	47.3±6.4	65.7±1.5	35.1±5.3	65.3±0.4
	% protein	14.0±0.3	13.3±1.1	15.1±0.2	14.2±0.5
Mature	% solubles	0	0	0	0
	% starch	64.8±2.3	76.4±1.6	58.2±0.6	77.0±1.9
	% protein	13.7±0.2	12.8±1.4	15.7±0.2	10.9±1.1

Dissected endosperms were lyophylized for two days and ground to a fine meal. Solubles were determined by the mass that could be extracted from the flour with methanol. Starch and protein were determined as described in Spielbauer et al. (2009). Errors are standard deviation of three biological replicate measurements.

Table S4. $NADP^+$ and NADPH levels (nmol/g FW) in 21 DAP kernels.

	wt	*pgd3*
$NADP^+$	42.9 ± 1.1	33.3 ± 0.9
NADPH	13.1 ± 1.8	10.5 ± 1.8
NADP/NADPH	3.29	3.17

Whole kernels were assayed for metabolite levels using a G6PDH cycling assay as described in the Materials and Methods. Errors are standard deviations of three biological replicates.

Table S5. Metabolite ratios in 20 DAP *pgd3-umu1* and *pgd3-umu2* mutant and normal endosperms.

	pgd3-umu1/+		*pgd3-umu2/+*	
Metabolite	mutant	normal	mutant	normal
Amino Acids				
Alanine	-1.03 ± 0.22	1.00 ± 0.21	1.36 ± 0.28	1.00 ± 0.14
β-Alanine	-1.64 ± 0.08	1.00 ± 0.05	-1.45 ± 0.07	1.00 ± 0.05
Arginine	28.34 ± 11.96	1.00 ± 0.04	18.67 ± 12.43	1.00 ± 0.07
Asparagine	1.42 ± 0.15	1.00 ± 0.13	1.79 ± 0.09	1.00 ± 0.10
Aspartate	-1.87 ± 0.09	1.00 ± 0.05	-1.71 ± 0.09	1.00 ± 0.05
GABA	1.07 ± 0.05	1.00 ± 0.05	-1.02 ± 0.05	1.00 ± 0.05
Glutamate	-1.81 ± 0.09	1.00 ± 0.05	-1.69 ± 0.09	1.00 ± 0.05
Glutamine	2.33 ± 0.36	1.00 ± 0.17	1.85 ± 0.28	1.00 ± 0.12
Glycine	-1.20 ± 0.06	1.00 ± 0.05	-1.09 ± 0.07	1.00 ± 0.06
Histidine	2.62 ± 0.17	1.00 ± 0.08	2.95 ± 0.23	1.00 ± 0.07
Homoserine	-1.10 ± 0.12	1.00 ± 0.11	1.50 ± 0.11	1.00 ± 0.08
Isoleucine	-1.33 ± 0.06	1.00 ± 0.04	-1.23 ± 0.08	1.00 ± 0.06
Leucine	-1.11 ± 0.05	1.00 ± 0.04	-1.33 ± 0.08	1.00 ± 0.06
Lysine	n.d.	1.00 ± 0.05	n.d.	1.00 ± 0.04
Methionine	1.18 ± 0.05	1.00 ± 0.04	-1.26 ± 0.07	1.00 ± 0.05
Ornithine	-1.16 ± 0.06	1.00 ± 0.05	1.08 ± 0.09	1.00 ± 0.05
Proline	2.68 ± 0.14	1.00 ± 0.06	3.33 ± 0.21	1.00 ± 0.07
4-Hydroxy-Proline	10.14 ± 0.4	1.00 ± 0.08	11.52 ± 0.54	1.00 ± 0.05
Serine	-1.84 ± 0.11	1.00 ± 0.06	-1.67 ± 0.11	1.00 ± 0.07
Threonine	-1.78 ± 0.08	1.00 ± 0.05	-1.57 ± 0.07	1.00 ± 0.04
Tryptophan	1.75 ± 0.11	1.00 ± 0.04	-1.01 ± 0.04	1.00 ± 0.04
Tyrosine	-1.30 ± 0.04	1.00 ± 0.03	-1.28 ± 0.05	1.00 ± 0.04
Valine	-1.17 ± 0.06	1.00 ± 0.05	1.16 ± 0.06	1.00 ± 0.08
Organic Acids				
2-oxo-Gulonate	1.61 ± 0.36	1.00 ± 0.11	1.67 ± 0.43	1.00 ± 0.09
4-Hydroxy-Benzoate	-1.41 ± 0.06	1.00 ± 0.04	-1.50 ± 0.09	1.00 ± 0.06
Benzoate	-2.57 ± 0.15	1.00 ± 0.06	-2.48 ± 0.18	1.00 ± 0.07
Citrate	-1.31 ± 0.15	1.00 ± 0.11	1.60 ± 0.09	1.00 ± 0.10
Dehydroascorbate	-1.39 ± 0.16	1.00 ± 0.12	-1.13 ± 0.11	1.00 ± 0.10
Fumarate	3.33 ± 0.19	1.00 ± 0.04	1.65 ± 0.11	1.00 ± 0.03
Galactonate	173.07 ± 8.11	n.d.	157.16 ± 5.64	n.d.
Galacturonate	5.51 ± 0.44	n.d.	7.35 ± 0.61	n.d.
Glutarate	-4.83 ± 0.22	1.00 ± 0.05	-8.16 ± 0.37	1.00 ± 0.05
Glycerate	1.48 ± 0.09	1.00 ± 0.04	1.46 ± 0.14	1.00 ± 0.06
Malate	2.34 ± 0.15	1.00 ± 0.04	2.79 ± 0.10	1.00 ± 0.04
Phosphate	-1.69 ± 0.07	1.00 ± 0.04	-1.84 ± 0.11	1.00 ± 0.06
Pyroglutamate	-1.26 ± 0.06	1.00 ± 0.05	-1.65 ± 0.09	1.00 ± 0.05
Quinate	-1.78 ± 0.08	1.00 ± 0.04	-1.36 ± 0.06	1.00 ± 0.04
Saccharate	9.43 ± 0.93	1.00 ± 0.05	10.18 ± 0.50	1.00 ± 0.03
Succinate	1.25 ± 0.07	1.00 ± 0.04	-1.35 ± 0.05	1.00 ± 0.04
Threonate	-1.44 ± 0.08	1.00 ± 0.06	1.06 ± 0.04	1.00 ± 0.04
Fatty Acids				
Dodecanoate	-1.61 ± 0.23	1.00 ± 0.14	-1.48 ± 0.14	1.00 ± 0.09
Heptadecanoate	-2.89 ± 0.22	1.00 ± 0.08	-2.53 ± 0.28	1.00 ± 0.11
Nonanoate	-2.21 ± 0.06	1.00 ± 0.03	-2.72 ± 0.25	1.00 ± 0.09
Octadecanoate	-2.70 ± 0.19	1.00 ± 0.07	-2.44 ± 0.24	1.00 ± 0.10

Table S5 (continued). Metabolite ratios in 20 DAP *pgd3-umu1* and *pgd3-umu2* mutant and normal endosperms.

	pgd3-umu1/+		*pgd3-umu2/+*	
Metabolite	mutant	normal	mutant	normal
Sugars/Sugar derivatives				
2-amino-2-deoxy-Glucose	1.91 ± 0.23	1.00 ± 0.04	2.22 ± 0.22	1.00 ± 0.08
2-deoxy-Glucose	-2.18 ± 0.16	1.00 ± 0.07	-1.87 ± 0.19	1.00 ± 0.10
Erythrose	-1.16 ± 0.06	1.00 ± 0.05	-1.16 ± 0.04	1.00 ± 0.04
Fructose	2.96 ± 0.34	1.00 ± 0.04	2.65 ± 0.19	1.00 ± 0.05
Glucose	4.97 ± 0.23	1.00 ± 0.06	5.22 ± 0.22	1.00 ± 0.06
Isomaltose	6.62 ± 0.43	1.00 ± 0.05	6.02 ± 0.27	1.00 ± 0.05
Maltose	4.83 ± 0.37	1.00 ± 0.04	4.08 ± 0.23	1.00 ± 0.05
Melibiose	4.40 ± 0.26	1.00 ± 0.03	4.24 ± 0.24	1.00 ± 0.07
Melezitose	-1.01 ± 0.04	1.00 ± 0.04	-1.15 ± 0.07	1.00 ± 0.06
Raffinose	-1.03 ± 0.05	1.00 ± 0.05	-1.03 ± 0.05	1.00 ± 0.05
Ribose	1.17 ± 0.08	1.00 ± 0.04	-2.37 ± 0.23	1.00 ± 0.10
Sucrose	-1.68 ± 0.24	1.00 ± 0.14	-1.88 ± 0.19	1.00 ± 0.10
Trehalose	1.05 ± 0.08	1.00 ± 0.04	1.14 ± 0.06	1.00 ± 0.06
Xylose	-1.52 ± 0.07	1.00 ± 0.04	-1.26 ± 0.08	1.00 ± 0.06
Sugar Alcohols				
Erythritol	1.59 ± 0.08	1.00 ± 0.04	1.35 ± 0.05	1.00 ± 0.04
Galactinol	-3.84 ± 0.10	1.00 ± 0.03	-9.33 ± 0.49	1.00 ± 0.05
Glycerol	-1.66 ± 0.07	1.00 ± 0.04	-1.10 ± 0.06	1.00 ± 0.05
Inositol	-2.98 ± 0.16	1.00 ± 0.05	-3.51 ± 0.22	1.00 ± 0.06
Maltitol	3.52 ± 0.21	1.00 ± 0.06	3.53 ± 0.10	1.00 ± 0.06
Phosphates of Sugars and Sugar Alcohols				
Fructose-6-P	1.30 ± 0.10	1.00 ± 0.05	2.87 ± 0.23	1.00 ± 0.06
Glucose-6-P	-1.28 ± 0.10	1.00 ± 0.08	1.53 ± 0.10	1.00 ± 0.06
Glycerol-3-P	2.39 ± 0.13	1.00 ± 0.07	2.43 ± 0.07	1.00 ± 0.06
Inositol-1-P	-3.16 ± 1.26	1.00 ± 0.40	-3.57 ± 1.54	1.00 ± 0.43
Others				
Adenosine-5-P	1.12 ± 0.09	1.00 ± 0.06	1.23 ± 0.11	1.00 ± 0.08
Gluconate-1.5-lactone	74.03 ± 5.48	n.d.	69.93 ± 9.36	n.d.
Glucarate-1.4-lactone	7.15 ± 1.32	1.00 ± 0.32	11.01 ± 2.46	1.00 ± 0.32
Nicotinate	-1.23 ± 0.07	1.00 ± 0.06	-1.20 ± 0.07	1.00 ± 0.06
Putrescine	2.59 ± 0.22	1.00 ± 0.10	1.25 ± 0.07	1.00 ± 0.07
Urea	-3.21 ± 0.19	1.00 ± 0.06	-2.58 ± 0.10	1.00 ± 0.04
Uracil	-2.32 ± 0.14	1.00 ± 0.06	-5.89 ± 0.32	1.00 ± 0.05

Normal and *pgd3* endosperm tissues were dissected from *pgd3-umu1* and *pgd3-umu2* segregating ears and lyophilized for 2 days. The endosperm tissue was ground to a fine meal and extracted for metabolite profiling as in Witt et al. (2012). Relative ratios of each metabolite were calculated based on sample dry weight and % solubles (relative ratio = response ratio*100/sample dry weight/% solubles). The standard error of three biological replicates is given for each metabolite. n.d.= not detected.

Table S6. Ratios of selected isotopologues from [U-$^{13}C_6$]glucose kernel labeling experiments.

	111111/111000	000111/111000	110000/111000
W22, replicate 1	0.32 ± 0.01 (n = 2)	1.22 ± 0.02 (n = 2)	0.45 ± 0.01 (n = 2)
pgd3 homozygous ear	0.80 ± 0.05*	1.33 ± 0.04*	0.51 ± 0.06
W22, replicate 2	0.23 ± 0.04	1.16 ± 0.01	0.44 ± 0.02
pgd3/+ear; wt seeds	0.25 ± 0.01	1.16 ± 0.01	0.39 ± 0.02
pgd3/+ ear; *pgd3* seeds	0.43 ± 0.06*	1.22 ± 0.01*	0.45 ± 0.02
pgd1; pgd2	0.25 ± 0.05	1.18 ± 0.04	0.44 ± 0.02

Kernels were labeled with [U-$^{13}C_6$]glucose in cob tissue culture blocks; starch was purified after a 7 day labelling period; and starch was digested with the resulting glucose molecules subjected to ^{13}C-NMR spectroscopy. Relative isotopologue abundances were then calculated within individual samples. Isotopologues are denoted with a six digit number with a 1 indicating a ^{13}C and a 0 indicating a ^{12}C in each carbon position of the glucose molecule. Unless noted, values are averages and standard deviations of three biological replicates. An asterisk (*) indicates a statistically significant, p(t-test)<0.05, change in comparison to W22 kernels.

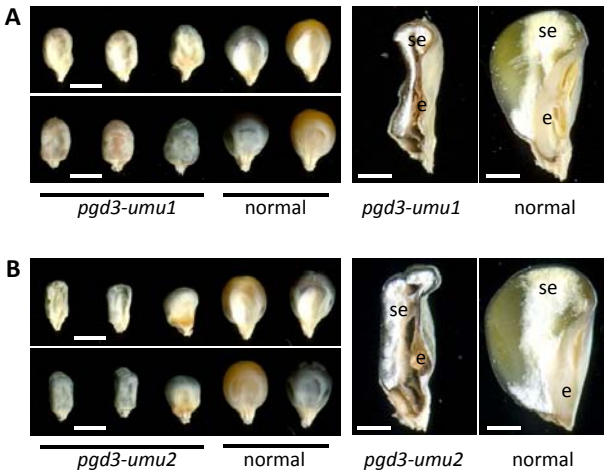

Fig. S1. Equivalent phenotypes for (A) *pgd3–umu1* and (B) *pgd3-umu2* alleles. Whole kernel images and sagittal hand sections of mature normal and *pgd3* mutant sibling seeds. Scale is 5 mm in whole kernel images and 2 mm in hand sections. se, starchy endosperm; e, embryo.

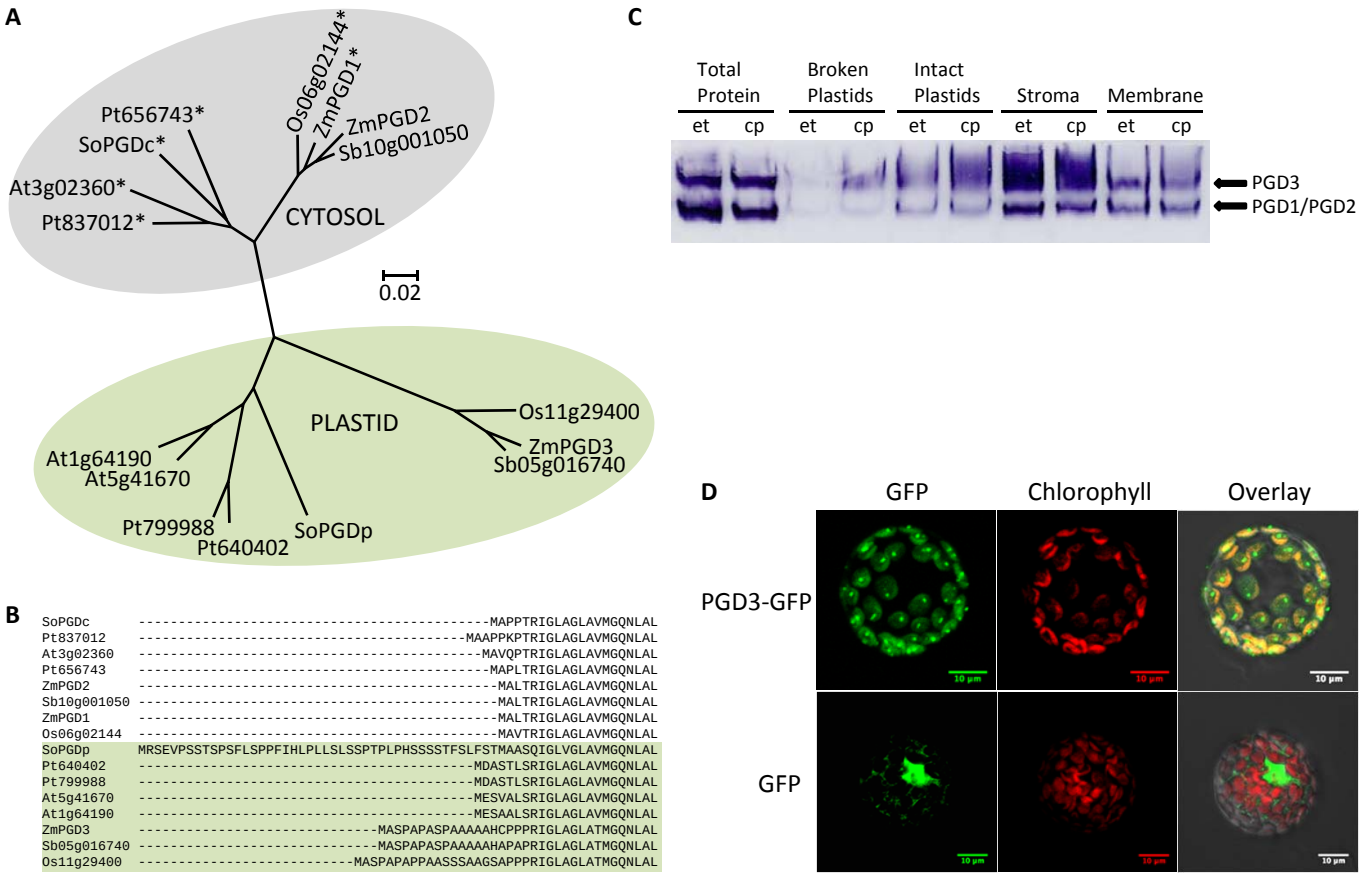

Fig. S2. PGD3 is a chloroplast-localized 6PGDH. (A) Sequence similarity tree of selected angiosperm 6PGDH enzymes. (B) Protein sequence alignment showing the N-terminal region of 6PGDH enzymes. The sequences were aligned and the neighbor-joining tree was constructed with Clustal X. A predicted PTS1 peroxisomal targeting motif is indicated by an asterisk (*). Species locus and accession numbers are: *Spinacia oleracea* (SoPGDc, AAK51690; SoPGDp, AAK49897); *Arabidopsis thaliana* (At3g02360, NP_850502; At1g64190, NP_176601; At5g41670, NP_198982); *Populus trichocarpa* (Pt837012, XP_002329247; Pt656743, XP_002311423; Pt799988, XP_002304540; Pt640402, XP_002297992); *Oryza sativa* (Os06g02144, AAL92029; Os11g29400, ABA93694); *Sorghum bicolor* (Sb05g016740, XP_002449496; Sb10g001050, none), *Zea mays* (ZmPGD1, ACN35899; ZmPGD2, AAC27703; ZmPGD3, ACG41643). (C) Association of 6PGDH enzyme activities with purified plastids from etiolated (et) and light-grown (cp) seedlings. Plastids were purified and subfractionated as described (Cline et al., 1993; Mori et al., 1999). (D) Transient expression of PGD3-GFP and GFP alone in Arabidopsis protoplasts. The overlay includes GFP, chlorophyll auto fluorescence, and a DIC image of the protoplast. The *Pgd3* ORF was amplified from cDNA synthesized using 1.5 µg total RNA of 12 DAP W22 seed tissue with the primers 5'-AAAAAGCAGGCTTCATGGCCTCCCCGGCGCCGGCC-3' and 5'-AGAAAGCTGGGTCAATGGCTGTGCCATTGCTCCT-3'. The RT-PCR product was recombined into the Gateway pDONR221 vector following the Invitrogen protocol. The ORF was transferred into p2GWF7 (Karimi et al., 2002) and transiently expressed in Arabidopsis protoplasts as described (Yoo et al., 2007). The GFP and chlorophyll signals were imaged 16 h after transformation with a Zeiss Pascal LSM5 microscope. Confocal images were processed with Image J 1.37v.

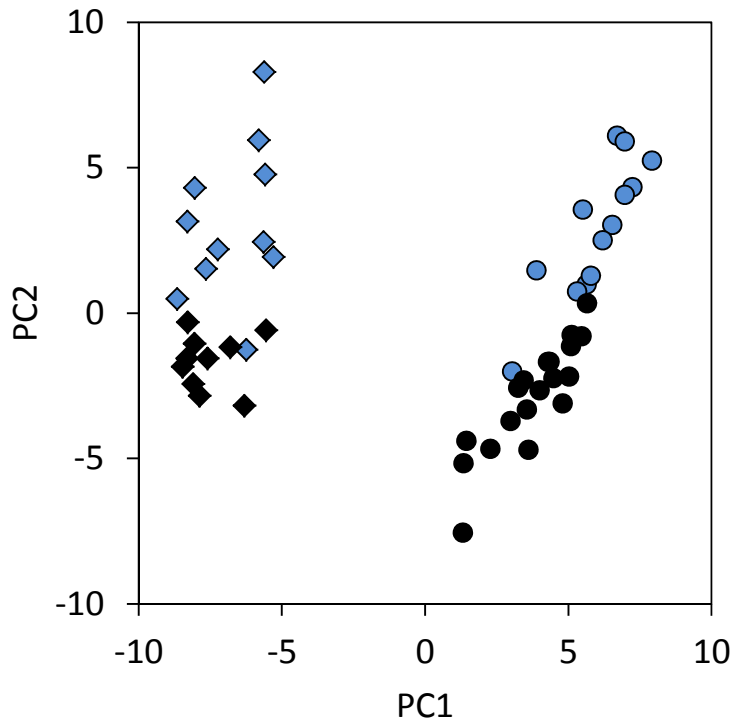

Fig. S3. Principal component analysis of normalized peak area ratios from endosperm metabolic profiles. Each symbol indicates a separate observation from an endosperm tissue. Diamonds indicate mutant and circles are normal siblings from ears segregating *pgd3/+*. Blue indicates kernels from *pgd3-umu1/+* ears and black indicates kernels from *pgd3-umu2/+* ears.